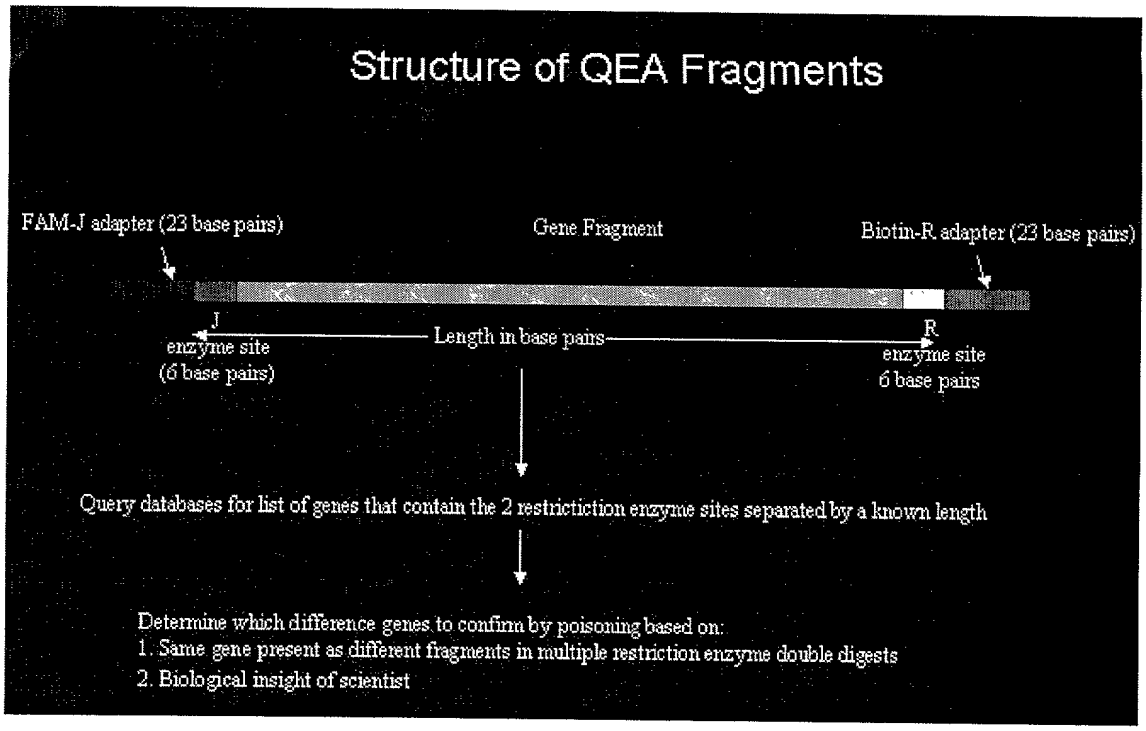

FIG. 1. Structure of QEA fragments. The FAM labeled J adapter (23 base pairs long) and biotin labeled R region (23 base pairs long) are oriented to the gene fragment as shown. The J enzyme site and R enzyme site are the restriction sites for their respective adapters (6 bp long). The larger central region plus the 2 restriction enzyme sites originate from a targeted nucleic acid sequence.

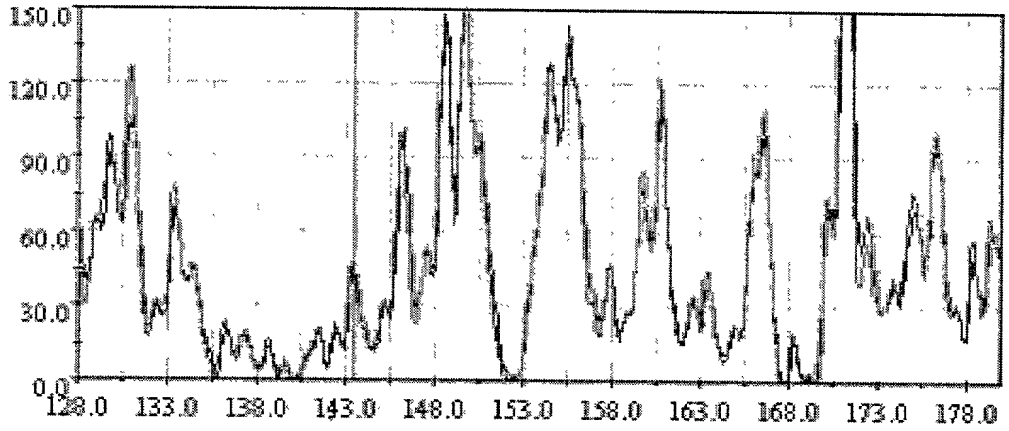

FIG. 2. Example of QEA peak traces from rat liver BglII BspHI double digest. Traces show peaks in the 120-180 bp region.

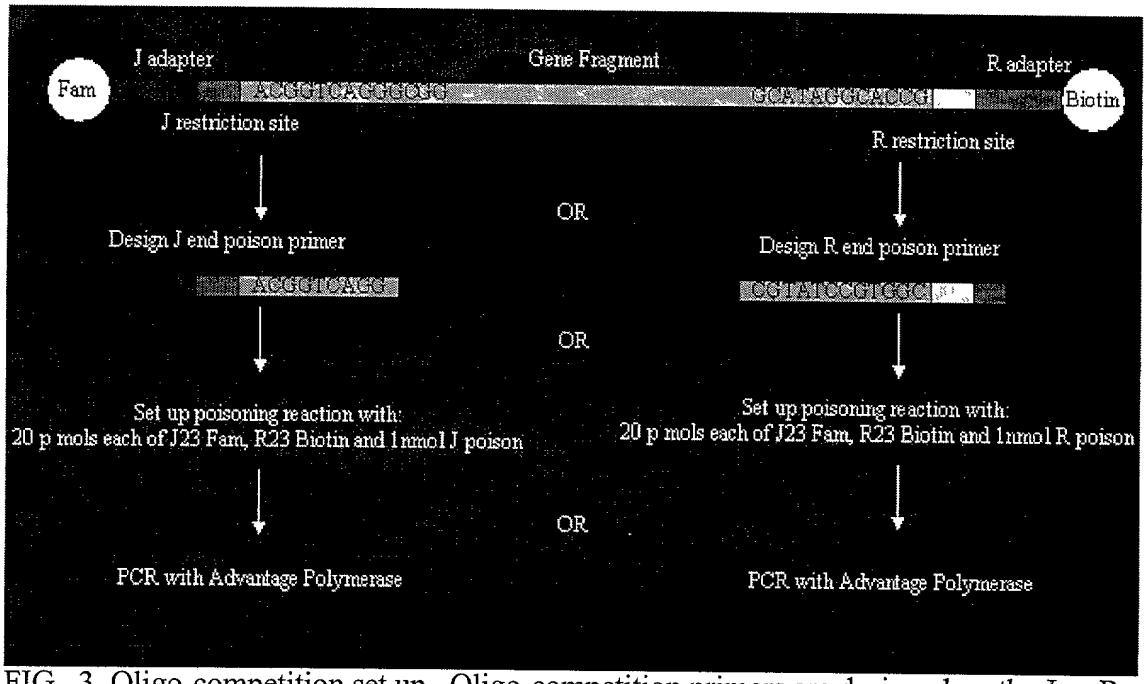

FIG. 3. Oligo-competition set up. Oligo-competition primers are designed on the J or R side based on the predicted sequence of the GeneCalled™ fragment. Oligo-competition reactions involve J23 and R23 primeres with a fifty fold excess of the oligo-competition primers.

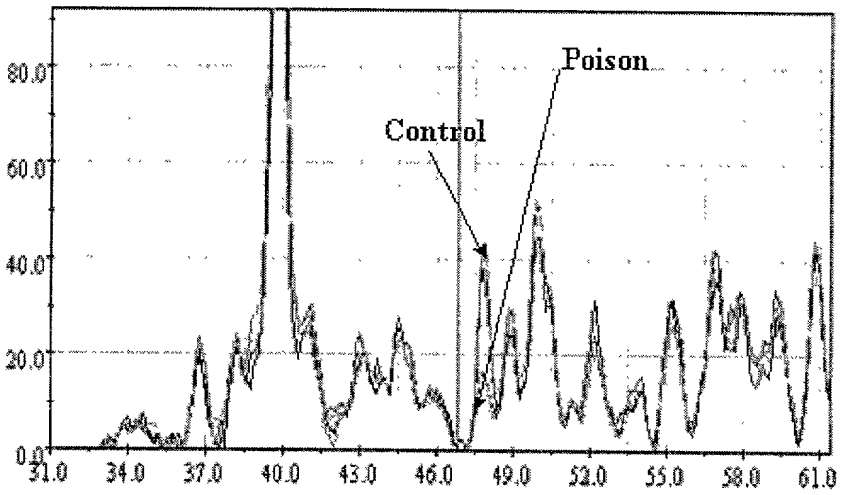

FIG. 4. Example of oligo-competition traces superimposed over control (no oligo-competition primer reactions) traces. (The traces are similar except where labeled. In this example the QEA peak at 48 bp was accurately sized, GeneCalled™, and poisoned.

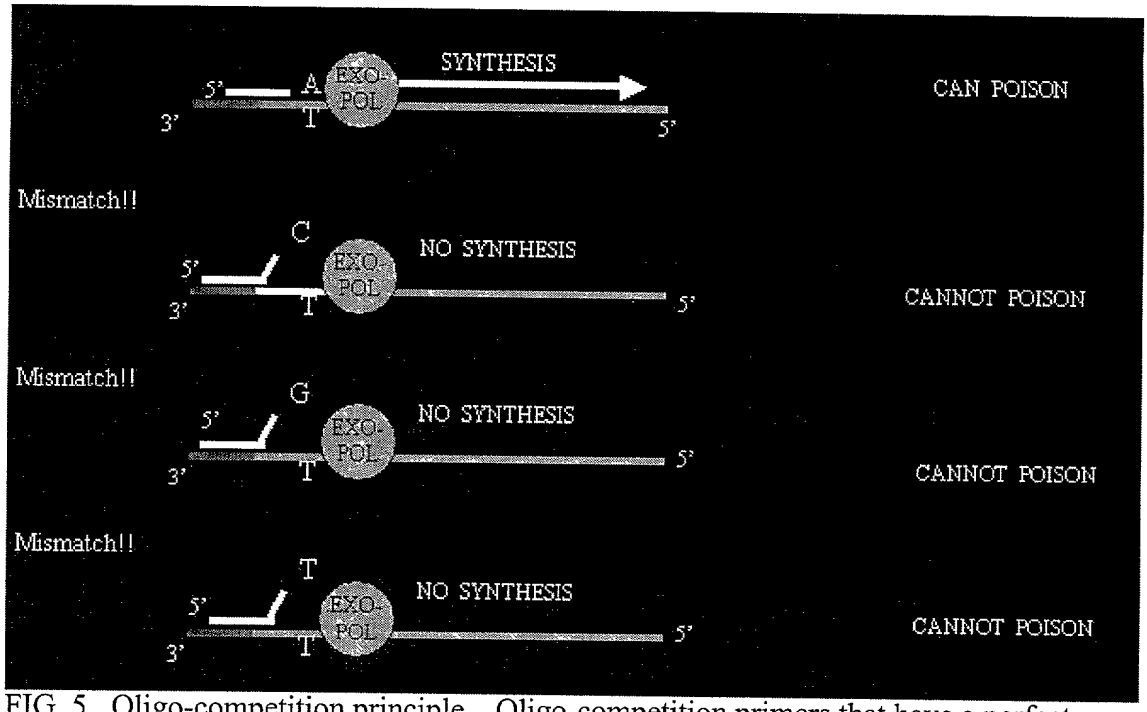

FIG. 5. Oligo-competition principle. Oligo-competition primers that have a perfect match at their 3' end with respect to the template strand are able to support DNA synthesis with an exonuclease-deficient DNA polymerase, and are therefore able to compete with J23 and R23 primers leading to the oligo-competition of these peaks. In contrast, QEA peaks with mismatches at the their 3' termini cannot support DNA synthesis, and will not be oligo-competitioned.

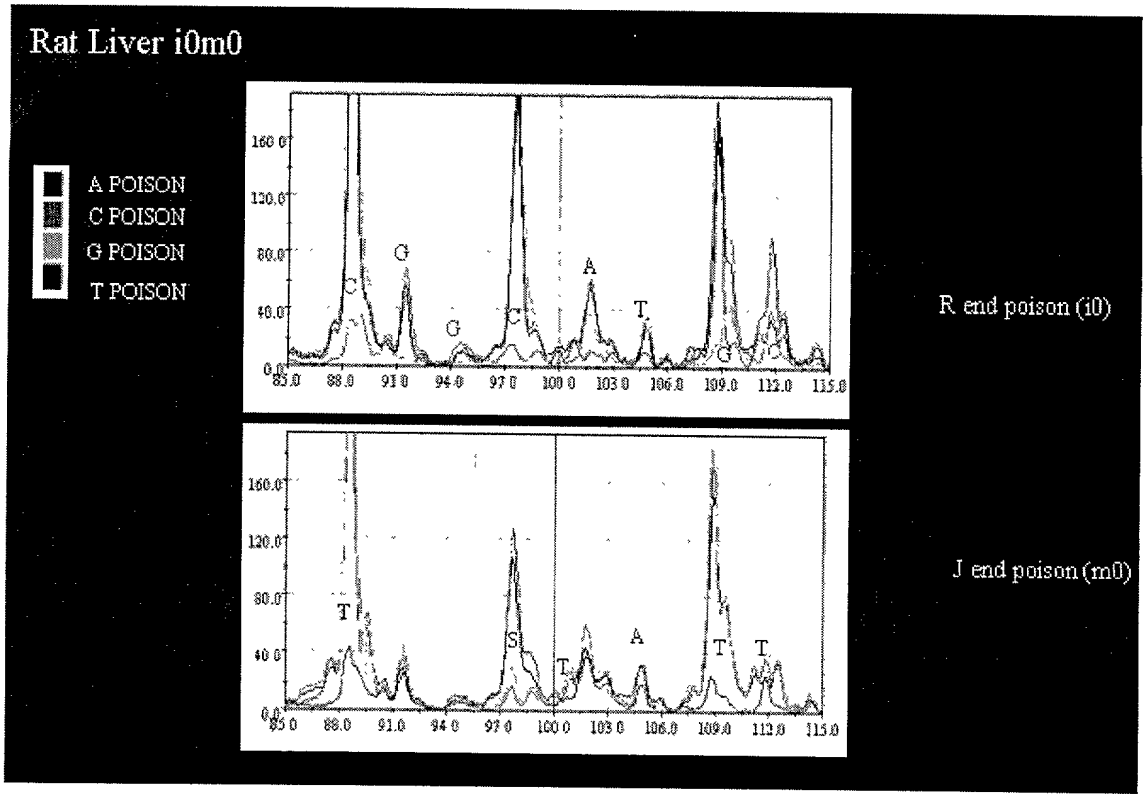

FIG. 6. Example of idnetification of the first base on the 3' side of the restriction enzyme sites on the R and J side for each QEA peak in the BsphI-BglII double digest of rat liver cDNA. (see text for details)

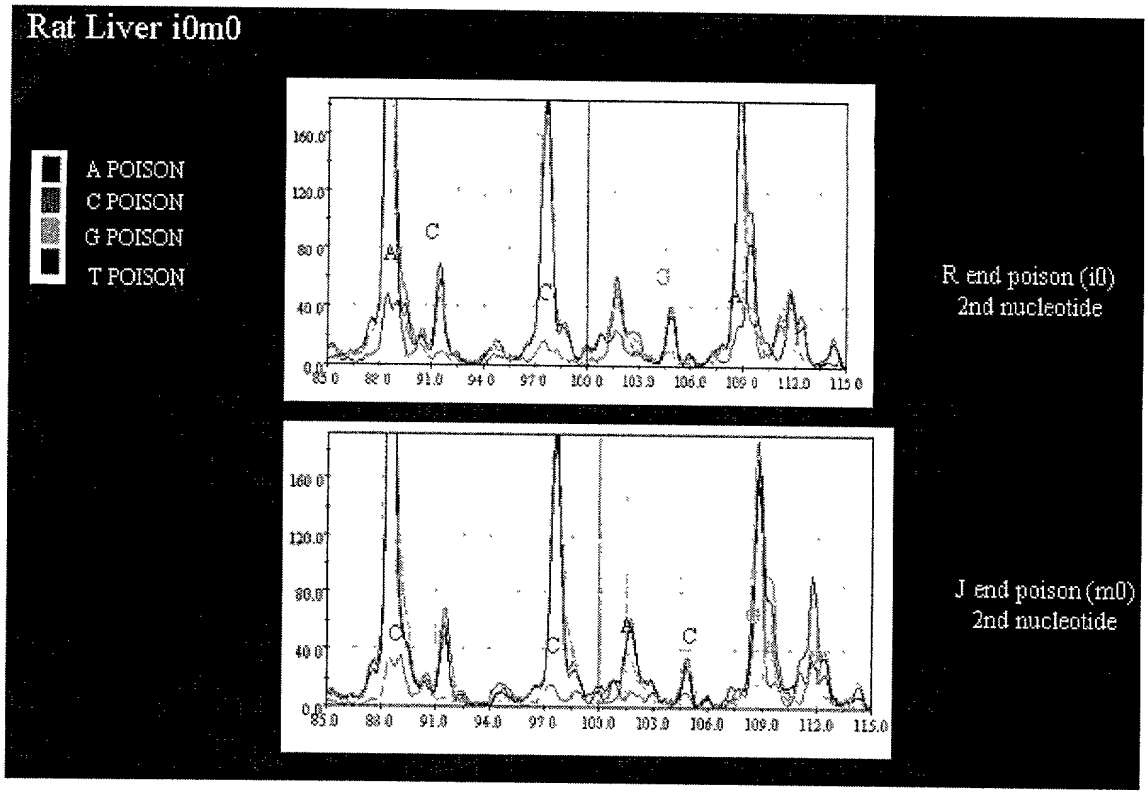

FIG. 7. Phasing traces for identification of the 2nd nucleotide on the 3' side of the BglII (top panel) and BspHI (bottom panel) sites.

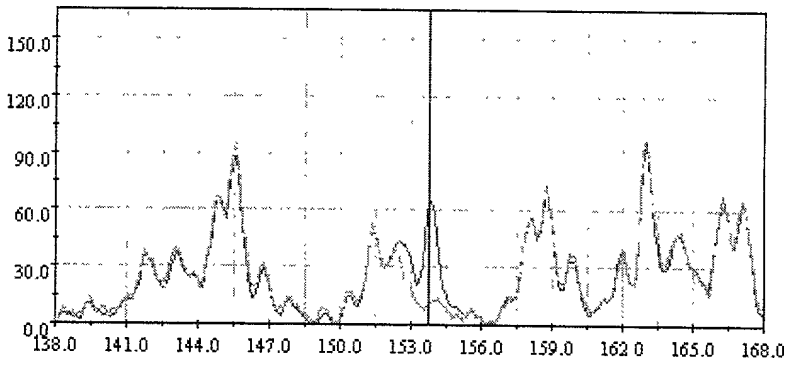

FIG. 8. Oligo-competition reaction (gray trace is the poison trace; black is the control trace) using a oligo-competition primer designed using the rat glycogen synthase gene confirms that GeneCall for the 153.8 bp BamHI-HindIII fragment.

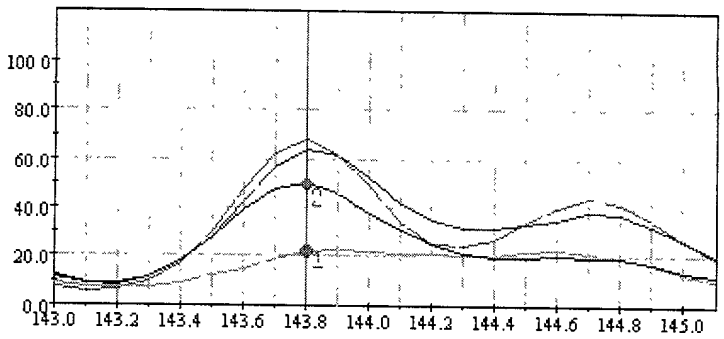

FIG. 9. Oligo-competition traces for a specific pair of cDNA cut sites, scaled and normalized intensity *vs.* fragment length, bp. One nucleotide is identified.

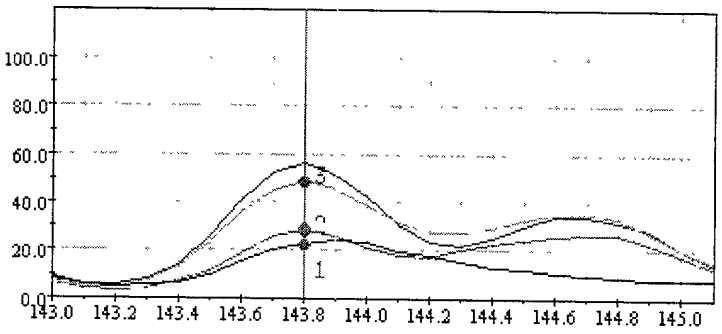

FIG. 10. Oligo-competition traces for a specific pair of cDNA cut sites, scaled and normalized intensity *vs.* fragment length, bp. Two nucleotides are identified.

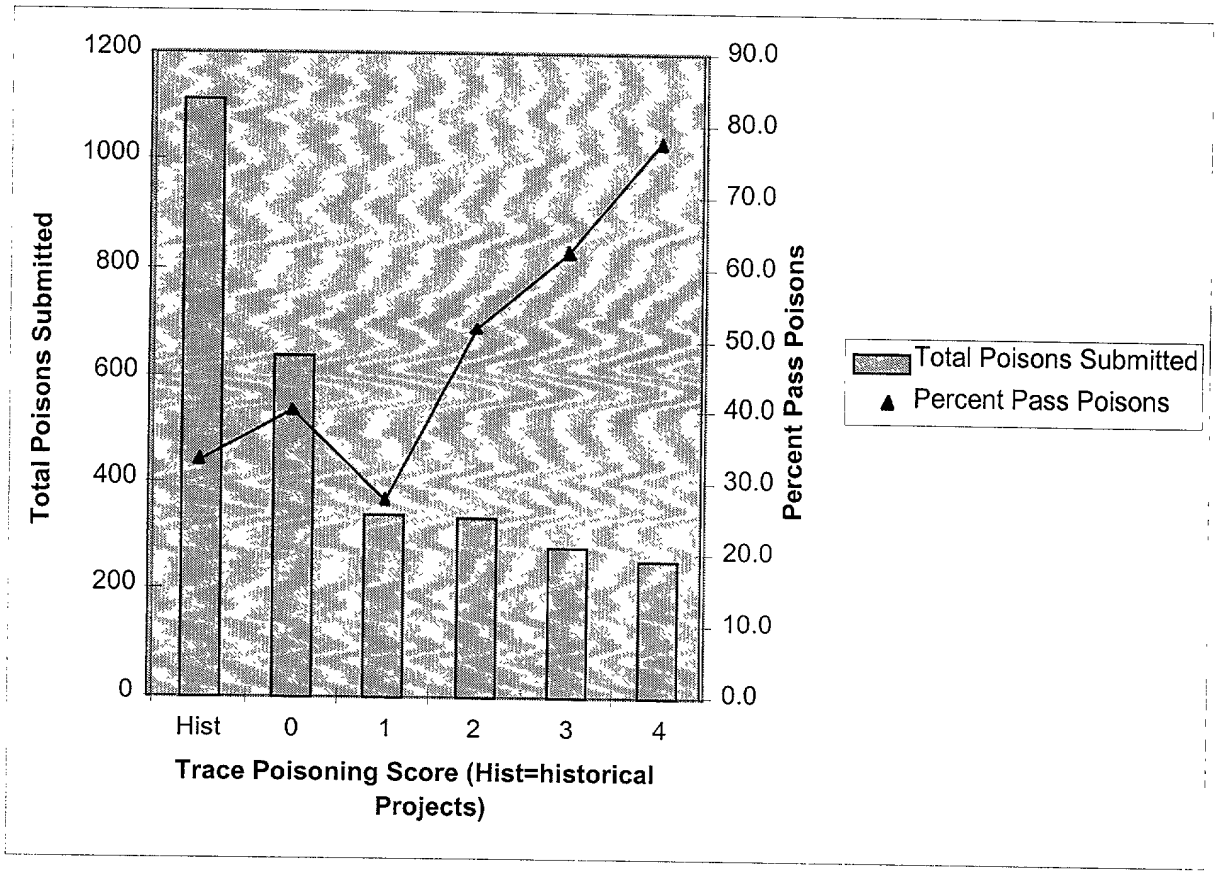

FIG. 11. Overall association of the trace oligo-competition score and trace oligo-competition effectiveness compared to historical data.

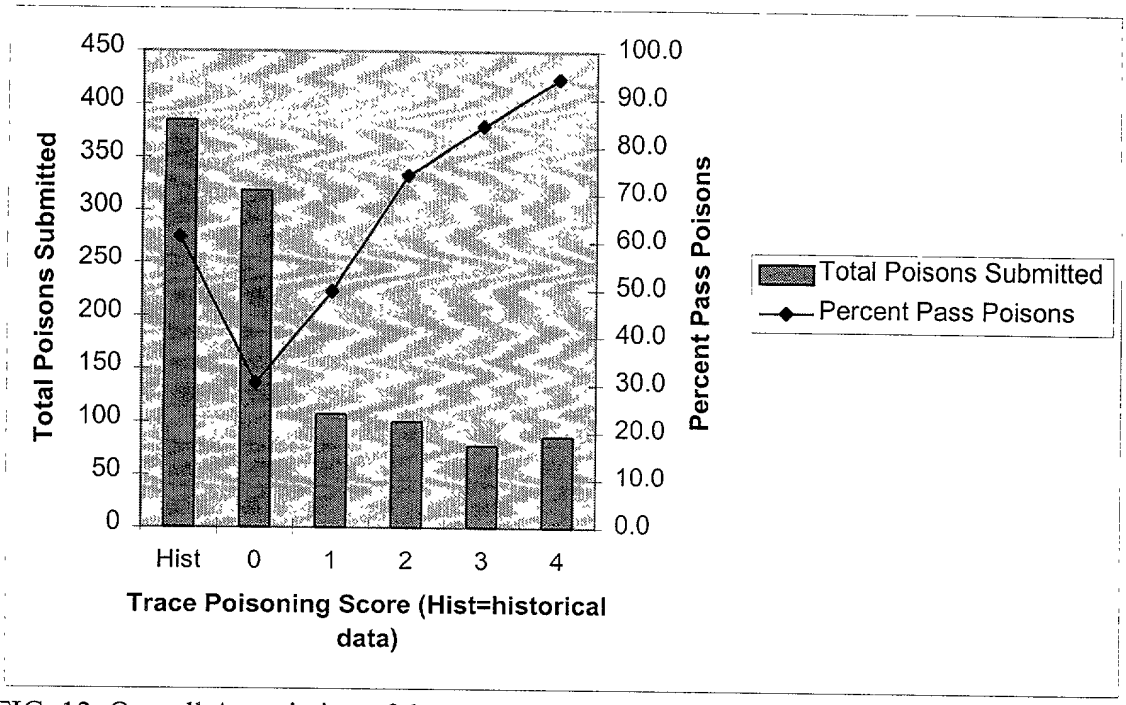

FIG. 12. Overall Association of the trace oligo-competition score and oligo-competition success among GeneCalls from Sized SeqCalling database.

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